SEPTEMBER 6.

Mr. CHARLES MORRIS, in the chair.

Sixteen persons present.

SEPTEMBER 13.

Mr. CHARLES MORRIS, in the chair.

Sixteen persons present.

SEPTEMBER 20.

Mr. THOMAS MEEHAN, Vice-President, in the chair.

Eighteen persons present.

SEPTEMBER 27.

Mr. JOHN H. REDFIELD, in the chair.

Eight persons present.

The death of Joseph Patterson, a member, was announced.

The following was ordered to be printed:—

ON THE HISTOLOGY OF SALPA.

BY DR. CHAS. S. DOLLEY.

In connection with a study of budding in *Salpa* by Prof. W. K. Brooks, I have undertaken to review the histology of *Salpa*. Thirty years ago Prof. R. Leuckart[1] in commencing a study of the same animal made the remark; "Seit fünfzig Jahren sind diese Thiere unzählige Male von Zoologen und Anatomen beobachtet worden, and doch ist das Studium derselben immer noch versprechend und lohnend für den Forscher," which is still true: and though it may at first thought seem presumptuous to expect any thing new from an animal which has been repeatedly investigated during the last eighty years, it was deemed best to go over the ground according to the improved methods of modern histological research. The work was mostly done in the Biological Laboratory of Johns Hopkins University, and completed in the autumn of 1884 in the Laboratory of the Zoological Institute in Leipzig.

The specimens used, collected by the United States Fish Commission in Vineyard Sound, were preserved in alcohol, and in chromic or picric acids.

The so-called "*Tunica externa*" or cuticle of *Salpa* is a secretion product of the ectodermal cells of the "*Tunica interna*." It is a hyaline, homogeneous, elastic material, showing at times, especially in young specimens, a faintly laminated appearance. This lamination is absent in the adult forms, where it is destitute of any appearance of structure, the clear field being broken only by minute granules, and occasional small stellate or spindle-shaped cell-like bodies scattered irregularly through it. These latter are, so far as I have been able to observe, destitute of nuclei, and have no connection with one another. They are probably the remains of cells which have wandered from the ectoderm into the newly formed cuticular secretion. Like the outer mantle of *Doliolum* and the "Haus" of *Appendicularia*,[2] it seems probable that this cuticle is from time to time shed and renewed. I assume this from having found in my collection several empty outer mantles, and also numerous specimens in which

[1] Rudolf Leuckart:—"Zoologische Untersuchungen." "Zur Entwickelungsgeschichte der Tunicaten. Salpen und verwandte." Giessen 1854.

[2] Basilius Uljanin:—"Fauna und Flora des Golfes von Neapel." Monographie X. *Doliolum*. p. 14. Herausgegeben von der Zoologischen Station zu Neapel, 1884.

the outer mantle was unusually thin and soft as if newly formed, while the exuviæ were stiff and elastic.

The "*Inner Mantle*" presents an ectodermal and an entodermal cellular layer, which are separated by a hyaline connective basis substance, of varying thickness, in which lie buried the viscera and the muscular bands, and through which a net-work of blood sinuses burrow in all directions.

The ectoderm consists of a single layer, of pavement epithelium, made up of polygonal, usually hexagonal cells (Plate xiii, figs. 1, 2), in which the protoplasm with its oval, often slightly bowed nucleus occupies the central portion; the remainder of the cell appearing empty and transparent, and the boundaries between neighboring cells being very poorly defined. These cells lie directly upon the basis substance of the inner mantle, and are on their outer surface in contact with the cuticle or outer mantle.

I have nowhere in the ectoderm of *Salpa* been able to find such large pavement cells, containing a protoplasmic reticulum extending out from a centre plasma-mass as Uljanin[1] and Grobben[2] described in the larvæ of *Doliolum;* but in several young specimens I find a layer of epithelial cells, lining the cavity containing the elæoblast which present an appearance corresponding in almost every particular to those described by Uljanin. The plasma of these cells is mostly collected into a central mass from which go out processes, anastamosing freely and connecting it with a thin, less granular layer at the periphery. The nuclei are oval in form and sometimes lie to one side of the central mass. Not having living specimens at hand, I was unable to ascertain anything in regard to the granular streaming in the protoplasmic network or the retraction of processes and the extension of others by the central mass as described by Uljanin. These cells lining the elæoblastic cavity are several times larger than the cells of the ectoderm covering the body, but they are similar in structure, and are probably larger because younger, since Uljanin found several undergoing fission. At the two openings of the body, branchial and cloacal, the ectoderm passes over into the entoderm or layer of cells lining the inner mantle; these correspond almost exactly with those of the ectoderm, except that the cells are usually from one third to one half smaller. They vary both in

[1] l. c. p. 13.

[2] C. Grobben:—*Doliolum* und sein Generationswechsel, Arbeiten des zo olog. Institutes zu Wien. Bd. IV, 11 ft. 2.

breadth and thickness in various parts of the body, as do the ectodermal cells. The entodermal cells are raised up into two ciliated bands upon the lateral walls of the branchial chamber and form a hoop-like elevation about its anterior end, which being inclined backward as it passes toward the dorsal surface, connects the anterior ends of the endostyle and the gill.

Between the ectoderm and entoderm is a transparent structureless material of the same appearance as that composing the outer material, but lacking the elasticity of the latter on account of its being pierced and hollowed out by the numerous blood channels and sinuses. Lying embedded in this porous matrix are the *Muscles;* these are composed of from six to twelve broad, flat, striated muscular fibres arranged in bundles, with their broad surfaces in contact and their edges presenting towards the interior and exterior of the body. (figs. 3, 4, 5.) The fibres are made up of several large muscle cells which have become fused together, each fibre showing a large number of oval nuclei, clear, bladder-like, with relatively large nucleoli. The fibres have a longitudinally striated appearance caused by the granular contents being arranged in rows representing the ultimate fibrillæ. The transverse striation is not always to be seen, but there is usually present, especially when the bundles are viewed on the surface, an irregular transverse marking of the entire bundle, due to certain portions of the cells taking a deeper staining. (fig. 5.)

The *Gill* ("hypopharyngeal band" Huxley) is a cylindrical tube in the living animal, but in preserved specimens more or less collapsed. Its walls are a continuation of the entoderm, and it is filled with the same spongy basis material that separates the ectoderm and entoderm, and like this it is perforated by an irregular series of blood sinuses; not by "a single grand sinus" as described by Huxley[1] (fig. 6, 7.). The cells constituting the walls of the gill are in the main, identical with those of the entoderm and remain unchanged along the upper and lower surfaces of the organ, but on the sides they become altered into two longitudinal series of ciliated ribs (*c b*, fig. 6.). These form cushion-like elevations, and are made up of three layers of spindle-shaped cells, the outer layer of which bear rather long stiff cilia.

The cilia-bearing cells are arranged in regular rows upon the cush-

[1] Huxley, (F. H.):—On the Anatomy of *Salpa* and *Pyrosoma*. Royal Society Transactions, 1851, p. 570.

ion, and the number constituting a transverse row in one of the cushions has been given by Leuckart[1] as from four to twelve; but I find a much wider variation than this in the same and in different individuals (fig. 7.). The number of ciliated ribs or cushions also varies greatly, running from one hundred to two hundred. At the anterior end of the gill two of the upper series of cushions are continued out upon the wall of the branchial sac, and form the semicircular ciliated bands, which run obliquely around the anterior extremity of the endostyle.

The *Endostyle* of *Salpa runcinata-fusiformis* (fig. 8) differs considerably from that which has been so thoroughly described by Fol[2] in various Tunicates, among others *Salpa maxima, S. bicaudata, S. pinnata, S. democratica.* Running along each side of the endostyle on the floor of the branchial cavity are the two ciliated border bands described by Fol (fig. 8, *cbb.*). In some specimens but one of these bands appears to be ciliated, causing a lack of symmetry as was early pointed out by H. Müller and recently by Seeliger. They consist of twelve or more thick cylindrical cells, bearing numerous cilia. These bands are separated from the endostyle proper, by a space of the ordinary pavement epithelium of the branchial sac. The "inner" (Fol) "upper" (Uljanin) glandular cushion is usually in section made up of thirteen or fourteen large columnar glandular cells poorly defined from one another (fig. 8, *igc.*). Those lying deepest in the groove are the longest and thickest, giving the cushion a wedge-shape, the apex forming the upper edge of the endostyle. The cells of this cushion have a granular contents and present in the lower third large bladder-like nuclei with relatively large and striking nucleoli; the upper portion of each cell is coarsely granular, and it is difficult to say where the cell ends and the secreted matter begins. Usually the two cells lying at the lower end of the cushion show a longitudinal striation composed of dark bacillus-like spots. According to Seeliger these cells contain the pigment particles, probably the coarse granules of my preserved specimens, which gives to the endostyle its blue color. There is no "middle intermediary band" present in the endostyle of *Salpa runcinata-fusiformis*, the "inner glandular cushion" resting directly upon the "middle glandular cushion" (*igc.* and *mgc.* fig. 8.). This is also the case with the

[1] l. c. p. 36.

[2] *Fol.* (*Hermann*):—Ueber die Schleimdruse oder den Endostyl der Tunicaten. Morphologisches Jahrbuch. Vol. 1. 1876.

specimens described and figured by Seeliger. The middle glandular cushion consists of eleven long pear-shaped cells, arranged with their large ends towards without, giving a section of the cushion a kidney-shape. The contents of these cells have none of the coarsely granular look of those of the "inner" glandular cushion, but present a faint longitudinal striation; they bear very clear round nuclei in their basal portions, in the centre of which are prominent round nucleoli. Below the "middle" glandular cushion is the so-called "outer intermediary band," (fig. 8, *oib.*) but this is again different from any described by Fol. Instead of consisting of simple pavement cells it is here made up of three layers of spindle-shaped cells with long rod-like nuclei; the inner layer of cells bearing fine short cilia. The "outer intermediary band" as figured by Seeliger, differs from those of Fol and myself in being composed of non-ciliated *columnar* cells. The "outer glandular cushion" (*ogc.* fig. 8) is composed of eleven cells very like those of the "middle" glandular cushion, and arranged after the same manner, pear-shaped with large ends towards without. They present however several nucleoli in each nucleus instead of but one. The two halves of this cushion forming together the floor of the endostyle present their largest curves in an exactly opposite manner to that figured by Fol, but similar to that of Seeliger. The basal portions of the largest cells of the two halves lie together in place of being turned away from one another. In none of my specimens have I been able to find the exceedingly long cilia, nor the two small oval cells described by Fol as bearing them, and as lying between the right and left halves of the "outer glandular cushion."

The *Alimentary canal* begins with a trumpet-shaped pharynx (*ph* fig. 9,) the everted edges of which pass over into the entodermal lining of the inner mantel. Its cells are rather long, cylindrical and hyaline, with small clear nuclei in the basal portions; they bear coarse lancet-shaped cilia. The character of the cells remain the same throughout the œsophagus, (*oe* fig. 9) which is considerably contracted in diameter; but upon reaching that portion of the canal which corresponds to the stomach of *Doliolum*, although there is no special dilatation of the canal, they lose their cilia and assume a somewhat more cuboidal character appearing at times to be piled loosely upon one another in several layers. Just here where the ciliated epithelium of the œsophagus changes into that of the stomach, the alimentary canal is joined by two cœcal appendages, one

on each side lapping over the stomach and intestine (fig. 10 *cæ.*). The cells and their arrangement in these cœca are of an entirely different character from those of the œsophagus, stomach or intestine. A transverse section shows a series of very prominent, coarsely granular, pyramidal cells, containing in the lower third a round nucleus and several, usually three, nucleoli. They are separated from one another by lighter, finely granular spaces, which when viewed from the surface of the cœcum (fig. 11.) present a reticulated appearance.

Whether this is due to the presence of small polygonal cells separating and surrounding the large glandular cells, or whether it is a mesh work of threads, formed from the secretion of these cells and connecting them, as in the livers of some molluscs, I can not be positive; I can not, however, detect any nuclei in the cell-like spaces.

Huxley[1] described but one cœcal appendage in *Salpa*, and called it the stomach, into which, according to his description, opened the duct from the net-work of anastamosing tubules which ramify over the visceral nucleus. In the form which I have examined there are present two cœcal appendages (fig. 10) as single sections plainly show and as I have learned positively by a model of the visceral nucleus constructed according to Born's "Platten-Modillir" method.[2] Seeliger in his paper, referred to further on, also mentions and figures but one cœcum. I can only account for the disagreement between the observations of Huxley and Seeliger on the one hand and my own on the other, by the supposition that the number of cœca varies in different species. I shall take advantage of the earliest opportunity, however, to examine the visceral nuclei of all species of Salpæ.

My observation agrees with those recently made by Seeliger in confirming H. Müller's statement that no food is ever found in these cœcal appendages, but their lumen is often filled with a structureless product of secretion. Opening as they do at the anterior end of the stomach they are evidently of some material use in digestion, and from the arrangement and structure of their walls I am of the opinion that they function as hepatic organs, as was first proposed

[1] l. c. p. 571.

[2] Archiv. f. mikroscop. Anat. xxii, p. 584, 1883. Amer. Naturalist, April 1884.

by H. Müller[1] from the peculiar contents of the glandular cells as observed by him.

As set forth at length in a former communication to this Academy[2] I was unable in any of my specimens to find in the œsophagus or stomach of *Salpa* the large plasmodium described by Koratneff.[3] I have serial sections from many specimens showing the entire curve of the alimentary canal from the mouth to the rectum in which the lumen throughout is perfectly free from any organized protoplasmic mass. Other preparations show the food laden mucus passing from the branchial cavity through the pharynx, œsophagus and stomach. Before reaching the stomach, on account of its containing much protoplasmic material which has not yet been acted upon by the digestive juices, this mixture of food and mucus takes staining very well; but after reaching the stomach it gradually refuses to stain, and in the intestine consists of a mass of colorless debris, owing to the organic materials having been removed by digestion. Sections cut diagonally across the œsophagus sometimes appear as though the lumen of the tube was almost obliterated except a narrow slit on one side, this is not actually the case as sections cut longitudinally (fig. 9.) or at right angles prove.

My observations opposed to the presence of an inter-cellular or parenchymatous digestion in *Salpa*, referred to above, have since been confirmed by Oswald Seeliger[4] in his interesting and valuable paper on the budding of *Salpa*, in which he refers to this subject as follows:—"Ich muss demgegenüber nun darauf aufmerksam machen, dass Korotneff im Magen von *Salpa africana* Verhältnisse angetroffen hat, die darauf schliessen lassen, dass in denselben thatsächlich Nahrung hineingelangt und daselbst verdaut oder resorbirt wird. Ich habe leider Koratneff's Angaben über die "freien Magenzellen" und die "parenchymatöse Ernährung" der Tunikaten nicht an verschiedenen Objekten nachstudiren können, bei *Salpa democratica* aber fand ich sie nicht zutreffend. Auch von anderer Seite (Dolley)

[1] Heinrich Muller, Ueber die anatomische Verschiedenheit der zwei Formen bei den Salpen. Ztschr. f. wiss. Zool., Bd. IV. 1853, p. 330.

[2] "Some observations opposed to the presence of a parenchymatous or intracellular digestion in Salpa." April 15th 1884 vid. Proceedings, Acad. Nat. Sci. Philadelphia. Zool. Anz. 1884, p. 705.

[3] Dr. A. Koratneff. Ueber der Knospung der Anchinia. in Ztschr. f. wiss. Zool. Bd. 40. Hft. 1, 1884, Feb. 19.

[4] Oswald Seeliger. "Die Knospung der Salpen" Jenaische Zeitschr. fur Naturwissenschaft," Bd. 19, page 631.

hat man Korotneff's Auffassung nicht zustimmen können, und es wäre in der That eine nochmalige Nachprüfung von dessen Mittheilungen wünschenswerth, bevor man das eigenthümliche Verhalten der Tunikaten beim physiologischen Processe der Ernährung zu der Schlussfolgerung—der ich aber aus anderen Gründen volkommen beipflichte—verwendet "deswegen haben wir in unserem Falle Ursache, noch an der hohen genetischen Stellung zu zweifeln, die den Tunikaten zugeschrieben ist."

At the beginning of the intestine proper the cells composing the walls resume the appearance of those of the œsophagus and again cilia show themselves.

The presence of cilia for moving on the intestinal contents is necessary on account of the lack of any musculature in connection with the visceral nucleus. There is present under all the cells of this tract a delicate basement membrane in which nuclei may occasionally be seen. Spreading over the visceral nucleus is a net-work of delicate tubes; the "darmumspinnende Drüse" of Seeliger and others.

These consist of an extremely thin basement membrane bearing cuboidal cells of a pale transparent character in which there was no nucleus visible. In no place could I detect cilia in these tubules as described by Chandeleon[1] for *Perophora*, but I did find numerous large concretion-like masses of a dark-brown color. Seeliger believing these glandular tubules to be hepatic in function might perhaps consider the above dark-brown masses as biliary secretion.

The disadvantage of having no living specimens to examine is apparent when I say I can not find the plasma off-shoots from the stomach cells into the lumen of the same, nor the glandular cells, containing yellow drops, seen by Seeliger in the stomach walls of living specimens.

Filling up the cavity produced by the doubling of the intestine, and by the two cœcal appendages, lie the *Testes* which consists of a number of delicate tubes in which a basement membrane is scarcely apparent; and a layer of clear round cells containing pear-shaped nuclei, form the walls. I could not find the "spindle-shaped nucleated cells forming a sort of connective tissue about the gland as described by Leuckart.[2]

The *Heart* lies in a pericardium which appears to be but a sac

[1] Th. Chandeleon :—Recherches sur une annexer du tube digestif des Tuniciers in Bull de l'Acad. Roy. de Belgique. 44me Année. 2e Ser. T. XXXIX, p. 911, 1875.

[2] l. c. p 36.

formed from the entodermal layer of the inner mantle, the cells of both being similar in every respect. The heart itself is composed of a structureless basement membrane supporting a layer of striated fusiform muscle cells. These "fibre cells" lie upon their flat sides, with their long axes at right angles with the long axis of the heart. They have a single oval nucleus and present a delicate transverse striation (fig. 12.). They are much smaller than the fibres of the trunk muscles.

The *Elæoblast* (statoblast of Vogt.) situated on the central side of the body near the cloacal opening consists in hardened specimens of a mass of large irregularly polygonal bodies, showing no nuclei and varying greatly in size. They are opaque and have a coarsely granular appearance as if filled with a sort of yolk material. They are undoubtedly cells which have been greatly engorged and modified. In sections they usually drop out to a large extent owing to the removal of the oil and fat by the reagents, leaving a reticulum made of the transparent connective material of the inner mantel in a cavity of which, or rather on the outer side of which, the elæoblast lies; the entire mass being covered with the peculiar large plaster cells previously described.

The function of this body seems still to be undetermined. In adult specimens it disappears entirely, but is present both in solitary and chain *Salpæ* when young. Vogt held it to be homologous with the placenta, Salensky[1] considers that in those *Salpæ* developed from the egg it arises from the same elements out of which the blood corpuscles and muscles are formed ("amœboid follicular cells"); in the chain *Salpæ* it is developed from the mesoderm.

The *Nerve Ganglion* or brain presents a nearly spherical mass covered with a delicate membrane which seems continuous with the outer sheath of the nerve trunks. Upon section it shows an outer layer of apolar ganglion cells, only the nuclei of which are to be seen as a rule, and a central portion of lighter colored fibrillar ("punct") substance (fig. 13, *NG.*).

Resting upon the brain and in fact a continuation both of the central fibrillar core and the external layer of ganglion cells, is the *Visual Organ* of *Salpa*, (regarded by Huxley as an auditory organ.) Outside of its nervous central portion (fig. 13 *vo*) is a layer of rather large cylinder cells (fig. 13 *pc.*) containing in their inner halves a

[1] Salensky (W.):—"Uber die Entwickelungsgeschichte der Salpen" in Zeitschr. f. wiss. Zool. XXVII 1877. Morphol. Jahrb. III p. 591.

round nucleus and a quantity of dark pigment; the upper and outer halves being clear and transparent. In no case did I find the nuclei in the clear outer portion, as figured by Seeliger (l. c. pl. xi, fig. 13). These pigment cells are in their turn covered with a layer of columnar cells, each of which contains a nucleus in its outer end. This layer does not seem continuous with the entodermal layer covering the brain, and is probably a modified portion of the ectodermal layer of the inner mantel. In one or two specimens which I had prepared without ascertaining their specific name, I found the eye to be much more flattened than in the figure given here, and divided up into several lobes.

From the above description, *Salpa* would seem to possess a sort of compound eye. Passing out from the central fibrillar portion of the brain, are several nerve trunks; from eleven to twenty-five pairs, which show a clear envelope with a dark granular axis. No fibrils are to be seen. The brain is covered by the entoderm which is, however, not in direct contact with it at all points.

Below and anterior to the brain the entoderm of the median dorsal surface is invaginated to form the *Ciliated Sac,* (l'hypophyse.) This structure as seen in other Tunicates has given rise to much discussion in regard to its function. Ussow[1] and Julin[2] regard this as a gland, Joliet[3] considers it to be olfactory in character. In *Salpa* it consists of a simple tube (fig. 13, *l'Hy.*) closed at the end next the ganglion against which it rests, and opening at the other end into the branchial sac. Its walls are made up of short thick columnar cells carrying heavy cilia. It, however, possesses no such peculiarities as the glandular cœca described in *Ascidia mammillata.*

LETTERING OF FIGURES, Pl. XIII.

(*bs*) blood sinus.
(*Br or br*) branchia.
(*cb*) ciliated cushion (in gill).
(*cbb*) ciliated border band.
(*ct*) cuticle.
(*coe*) cœcal appendages.

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[1] Ussow:—"Beitrage zur Kenntniss der Organization des Tunicaten." Moscow, 1876.

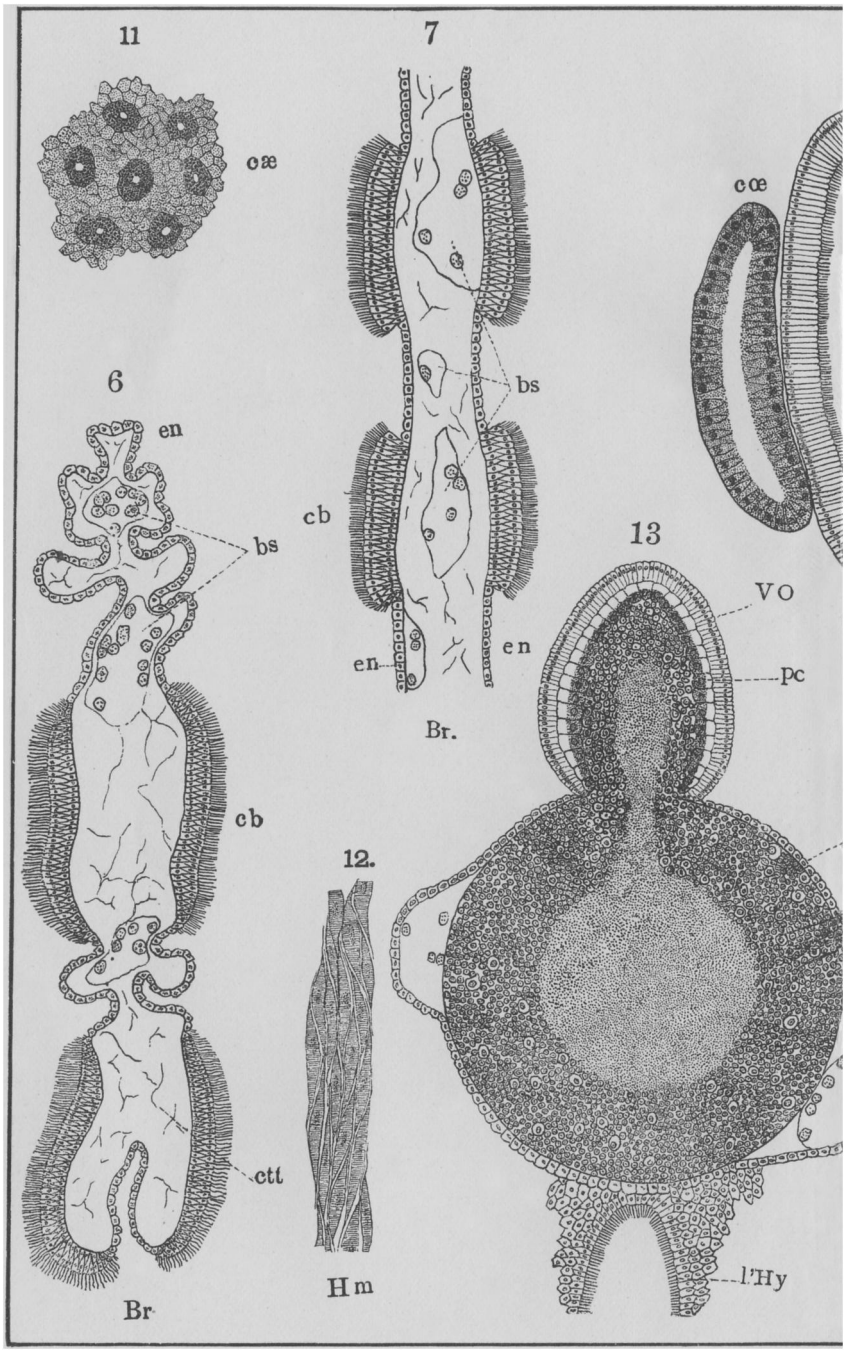
[2] Julin (Chas.):—l'Hypophyse des Ascidiens in Bull. Acad Sc. de Belgique, 3d. Ser. T. 1. P. 2, p. 151.

[3] Joliet. M. L.:—"Sur le developpement du ganglion et du "sac cilie" dan le burgeon du Pyrosome" Compte. rend., Ac. Sci. Paris. T. 94, No. 14, p. 988.

(*ec*) ectoderm.
(*en*) entoderm.
(*Hm*) heart muscle.
(*igc*) inner glandular cushion.
(*im*) inner mantle.
(*int*) intestine.
(*l'Hy*) l'hypophyse.
(*m. b.*) muscular bands.
(*mf*) muscles fibres.
(*mgc*) middle glandular cushion.
(*mib*) middle intermediary band.
(*nf*) nerve fibre.
(*ng*) nerve ganglion.
(*oe*) œsophagus.
(*ogc*) outer glandular cushion.
(*oib*) outer intermediary band.
(*pc*) pigment cells.
(*ph*) pharynx.
(*vo*) visual organ.

Explanation of Figures, Pl. XIII.

Fig. 1. Section of inner and outer mantles.
" 2. Surface view of ectoderm.
" 3. Portions of three muscle fibres from muscular bundle.
" 4. Section through one of the muscular bundles of the trunk showing its position in the inner mantle.
" 5. Seven muscle fibres as seen from a surface view of one of the bundles.
" 6. Transverse section of the entire gill.
" 7. Longitudinal section through a portion of the gill, on the plane of *cb.* in fig. 6.
" 8. Showing one half of a transverse section of the endostyle
" 9. Longitudinal section through the œsophagus.
" 10. Transverse section of the intestine and the two cœcal appendages.
" 11. View of the outer surface of a cœcal appendage.
" 12. Striated muscular "fibre cells" composing the wall of the heart.
" 13. Vertical section through brain, visual organ, and l'hypophyse.



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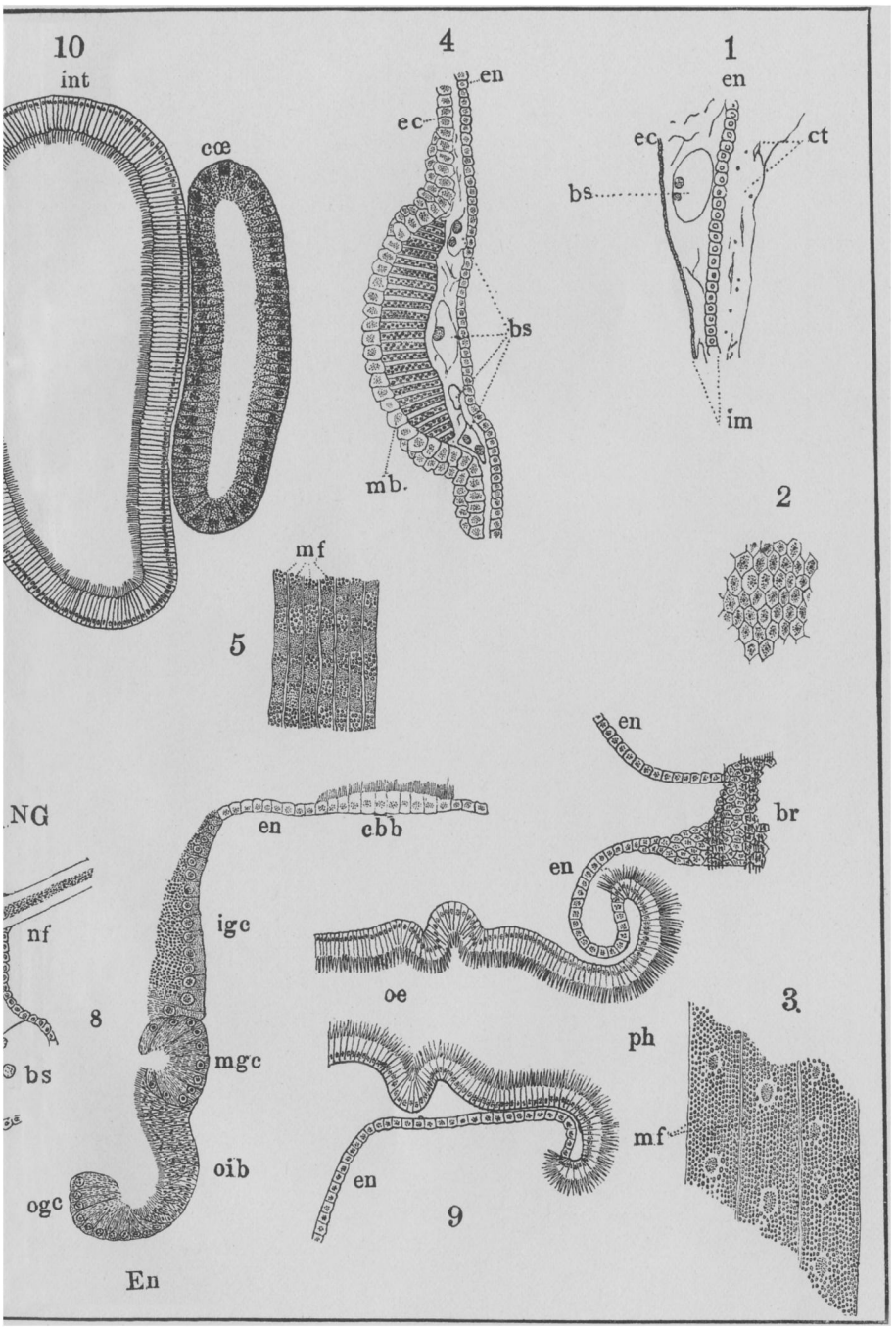
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ON SALPA.